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(54) Title: ALTERATION OF PHENOTYPE DUE TO HETEROLOGOUS GENES

(57) Abstract: The screenable phenotype of a target sequence in a host cell can be altered by fusing the target protein to a suitable fusion partner.

ALTERATION OF PHENOTYPE DUE TO HETEROLOGOUS GENES

Background of the Invention

The fundamental concept underlying the surrogate genetics target identification approach is the use of genetics to design assays for various types of human, animal, plant, viral, bacterial, and fungal biomolecules. This approach involves the creation of a variety of heterologous recipient cells that act as reporters of the activity of these biomolecules. The assays themselves serve as sensors to detect modulators that act directly or indirectly on the human, animal, plant, viral, bacterial, or fungal biomolecule. Both the assays and the target-hit correlation (compound "phenoprints") provide important insights into the relationships among genes, including how they work together within a metabolic pathway(s) and allows the best targets from a drug intervention standpoint to emerge from the screen.

As an example, it has been roughly estimated that in humans approximately 5,000 genes out of a total of approximately 100,000 genes could potentially serve as therapeutic targets. Of these, only a small fraction are currently being targeted. It is believed that many of the potential targets have not yet been identified. The present method thus enhances the efficiency and range of usage of potential targets by providing novel surrogate assays to characterize the activity of the gene, and a means to screen compounds for activity in a model system.

Some proteins are not well expressed in heterologous host cells, possibly due to differences in optimal pH, lack of accessory proteins for proper folding, abnormal degradation, and the like. Other proteins may produce a diagnostic phenotype in the host cell, but only at a low or barely-detectable level. As a result, not all proteins are currently ideal candidates for use in surrogate genetics assays.

Summary of the Invention

We have now invented a method for improving the phenotypic effect of heterologous proteins.

One object of the invention is to improve the phenotypic response of an Activity Reporter Cell ("ARC") that expresses a heterologous protein, thus enabling one to study heterologous proteins that would otherwise not be studied. Another object of the invention is to improve the

phenotypic effect of a heterologous protein (in general), so that one can more easily and accurately distinguish between inhibitors of the heterologous protein activity.

One aspect of the invention is a method for producing an assay cell having a screenable phenotype generated by a heterologous target gene in said assay cell, comprising introducing into a first host cell a first nucleic acid encoding a heterologous target protein operably connected to a promoter operable in said host cell; obtaining expression of said heterologous target protein; determining the degree to which said first host cell exhibits a screenable phenotype; in the absence of a screenable phenotype, introducing into said host cell a second nucleic acid encoding a fusion protein comprising said heterologous target protein fused to an enhancing fusion partner, operably connected to a promoter operable in said host cell; obtaining expression of said fusion protein; determining the degree to which said host cell exhibits a screenable phenotype; and selecting the host cell that exhibits a screenable phenotype to the greatest degree.

Another aspect of the invention is a method for producing an assay cell having a screenable phenotypic response generated by a heterologous target gene in said assay cell, comprising introducing into a first host cell a first nucleic acid encoding a heterologous target protein operably connected to a promoter operable in said host cell, and introducing into a second host cell a second nucleic acid encoding a fusion protein comprising said heterologous target protein fused to an enhancing fusion partner, operably connected to a promoter operable in said host cell; obtaining expression of said heterologous target protein and said fusion protein; determining the degree to which said first host cell exhibits a screenable phenotype and the degree to which said second host cell exhibits a screenable phenotype; and selecting the host cell exhibiting the greatest degree of screenable phenotype.

Another aspect of the invention is a process for identifying compounds capable of inhibiting the biological activity of a target protein, said process comprising: providing a first host cell comprising a first nucleic acid encoding a heterologous target protein operably connected to a promoter operable in said host cell, and providing a second host cell comprising a second nucleic acid encoding a fusion protein comprising said heterologous target protein fused to an enhancing fusion partner, operably connected to a promoter operable in said host cell; obtaining expression of said target protein and said fusion protein; determining the degree to which said first host cell exhibits a screenable phenotype and the degree to which said second host cell exhibits a screenable phenotype; selecting the host cell exhibiting the greatest degree

of screenable phenotype; contacting the selected host cell with a candidate compound; determining the degree to which the screenable phenotype of said selected host cell is modulated by the presence of said candidate compound; and selecting a candidate compound that reverses said screenable phenotype to a measurable degree.

Another aspect of the invention is an assay panel, comprising a plurality of different first host cells, each first host cell comprising a first nucleic acid encoding a heterologous target protein operably connected to a promoter operable in said host cell, and a plurality of second host cells, each second host cell corresponding to a first host cell and comprising a second nucleic acid encoding a fusion protein comprising said heterologous target protein fused to an enhancing fusion partner, operably connected to a promoter operable in said host cell, wherein for each target protein at least one of said first host cell and said second host cell which expresses said target protein exhibits a screenable phenotype.

Brief Description of the Figures

FIG. 1 schematically illustrates the preparation of a phenotype-enhanced fusion vector.

FIG. 2 is a map of plasmid pRS315_lacZ.

FIG. 3 is a data table showing growth inhibition for a variety of target nucleic acids, with and without a fusion partner.

FIG. 4 shows growth inhibition obtained with an HIV protease-GFP fusion protein, and reduction of the inhibition following mutation of the protease active site (D25A).

FIG. 5 is a table showing that maximum growth inhibition (phenotype) is obtained using a fusion partner.

FIG. 6 shows the results of host cell growth for HIV protease constructs, titrated with a known inhibitor (saquinavir). FIG. 6(A) shows the results of growth in 2% galactose (target protein expression induced), while FIG. 6(B) shows the results of growth in 2% glucose (target protein expression not induced). FIG. 6(A) also demonstrates that phenotype reversal with saquinavir is possible if the protease is expressed from a CEN plasmid (low copy number), but not from a 2μ-based plasmid (high copy number).

Detailed Description

Definitions:

The term "screenable phenotype" as used herein refers to a quantifiable characteristic of a host cell that (a) varies with expression of a heterologous protein, and (b) is related to a metabolic or signal pathway in the host cell. The screenable phenotype is due directly or indirectly to the biological activity of the heterologous protein. Suitable screenable phenotypes include any measurable change in metabolism, for example, without limitation, survival, growth time or rate (increase or decrease), alteration of gene expression, protein expression level, susceptibility to an added compound, growth in the absence of an otherwise-required compound or nutrient, altered morphology (e.g., from rounded to spindle-shaped), apoptosis, enhanced or inhibited growth under altered conditions (for example, at elevated or depressed temperature, pressure, ionic concentration, and the like), and the like. The degree to which a host cell exhibits a screenable phenotype can be determined by standard measurement techniques, selected in accordance with the phenotype displayed. A "greater degree" of screenable phenotype refers to a larger deviation from control. The enhancing fusion partner can provide a detectable label (for example, a fluorescent protein, an enzyme capable of catalyzing a chromogenic reaction, an antigen capable of binding a selected antibody, and the like) in addition to the screenable phenotype, which can be useful for selecting effectively transformed host cells. The detectable label cannot substitute for a phenotype that results from the biological activity of the target protein, however, one can substitute a reporter gene array as a surrogate for an actual phenotype.

The terms "target protein" and "heterologous protein" refer to a protein that is either foreign to the host cell in which it is expressed, or is expressed in a manner foreign to its normal expression in the host cell. For example, mammalian proteins are heterologous to a yeast host cell, as are native yeast proteins expressed as fusion proteins, expressed under the control of a foreign promoter, and the like. Target proteins are "different" if they differ in sequence by at least one amino acid. The terms "target nucleic acid" and "heterologous nucleic acid" refer to nucleic acids that encode a target protein.

The term "enhancing fusion partner" refers to a protein or polypeptide which, when fused to a heterologous target protein, increases the screenable phenotype produced by the target protein. For example, if a given host cell exhibits a growth inhibition of 50% when expressing

a selected target protein, expression of the target protein as a fusion protein fused to an enhancing fusion partner will produce a measurably greater degree of inhibition in the host cell. The degree of enhancement will depend on the target protein, the host cell, and the particular enhancing fusion partner selected, and on the type and degree of phenotypic effect achieved by the target protein alone. For example, if a particular target protein produces a growth inhibition phenotype of 90%, the amount of enhancement is limited. Conversely, if the unenhanced phenotype is a 20% growth inhibition, the enhancement may be several-fold. Inhibition can be measured as a reduction in growth rate, or more conveniently as a reduction in the number of cells produced by culturing for a specified time. Suitable enhancing fusion partners include, without limitation, lacZ, URA3, GFP, and the like.

The term "host cell" refers to any cell capable of expressing a selected target protein (and a fusion protein), and having a reproducible phenotype. Presently preferred host cells are eukaryotic cells, for example yeast such as Saccharomyces cerevisiae, S. pombe and the like, insect cells, or mammalian cells, such as CHO cells, BHK cells, and other cell lines, primary cell cultures, and the like. "Different" host cells are cells that have at least one substantial difference in their nucleic acids (other than the target gene construct), such as, for example, deletion or mutation of a particular gene in one of the cells, different nutritional requirements, and the like. The term "corresponding" host cells, as used herein, refers to host cells having essentially identical nucleic acids and target genes, differing only in the target nucleic acid expression or fusion. For example, one can construct an assay panel comprising a set of otherwise identical yeast host cells: a first host cell having a target gene construct, a second host cell having a target gene-GFP fusion construct, a third host cell having a target gene-URA3 construct, a fourth host cell having a control vector construct (lacking the target gene), and a fifth host cell lacking any vector (wt control): each of these cells are "corresponding" if the target gene is the same in each case.

The term "assay panel" as used herein refers to a set of host cell populations, each comprising a different target gene. Preferably, each host cell carries only one target gene.

The term "candidate compound" refers to a substance which is potentially capable of affecting the biological activity of a target protein. Suitable candidate compounds include small organic molecules, proteins, peptides, lipids, nucleic acids, oligonucleotides, carbohydrates, natural products, cell extracts, and the like.

The term "promoter" as used herein refers to a nucleic acid that functions in the host cell to cause expression of an adjacent nucleic acid. Promoters can be regulated or constitutive. A heterologous nucleic acid is "operably connected" to a promoter if activation of the promoter results in expression of the heterologous nucleic acid.

General Method:

The methods of the invention serve as an extension of surrogate genetics methods and other assays in which a heterologous nucleic acid or protein is used to induce a phenotype in a selected host cell. In the practice of such methods, it is convenient to develop a "standard" assay cell line, or a panel or array of such cell lines, that provide a reproducible readout in response to a heterologous polynucleotide or protein. The readout can include any detectable behavior or characteristic, for example, survival, growth time (increase or decrease), alteration of gene expression, protein expression level, susceptibility to an added compound, growth in the absence of an otherwise-required compound or nutrient, altered morphology (e.g., from rounded to spindle-shaped), apoptosis, enhanced or inhibited growth under altered conditions (for example, at elevated or depressed temperature, pressure, ionic concentration, and the like), and the like. Alternatively, one can engineer assay cells to express a label in response to a particular alteration, for example by providing a panel of cells having a gene encoding a marker protein such as Green Fluorescent Protein (GFP) or a surface antigen operatively associated with an endogenous promoter or regulatory region.

The "surrogate genetics" approach relies on the fact that expression of a heterologous nucleic acid in a host cell is likely to cause some degree of interference with one or more metabolic or signal transduction pathways within the host cell, and that this interference can manifest as a detectable phenotype. However, not all heterologous proteins quickly provide such a phenotype. This may result from the availability of alternate signal or metabolic pathways that are unaffected by the heterologous protein, or due to instability of the heterologous protein in the host cell, or due to sequestration of the heterologous protein in a cellular compartment separate from the host proteins and/or nucleic acids with which it would interfere, or due to an inability to express a sufficient amount of the heterologous protein. These problems can sometimes be overcome by transferring the heterologous nucleic acid to a different cell, or by crippling additional pathways within the host cell, or by other techniques: however, such strategies may

require re-standardizing the assay cells used, or otherwise re-characterizing the resulting modified or alternate host cell, and require some knowledge of the function of the heterologous gene in order to engineer a specific modification (generally unavailable in the case of new, uncharacterized genes). The additional experimentation required results in delays and lost time. Further, even if alternate cells are readily available, the loss of some cell lines reduces the diversity of the assay panel, and thus reduces the amount of information that can be obtained regarding the heterologous protein.

The method of the invention provides a general technique for increasing the phenotypic effect of a heterologous target protein, without requiring other alteration of the host cell or selection of a different host cell. In the practice of the claimed method, an enhancing nucleic acid is inserted adjacent to the heterologous nucleic acid, so that upon expression the resulting product is a heterologous protein fused to an enhancing fusion partner. The enhancing fusion partner is preferably a protein of moderate size, having a high degree of structural stability, and can optionally comprise a detectable label. Exemplary enhancing fusion partners include, without limitation, lacZ, GFP, URA3, drug resistance markers, and the like. The enhancing fusion partner nucleic acid can be introduced at any time: it is presently preferred to prepare heterologous nucleic acids with and without the enhancing fusion partner simultaneously, thus providing two "versions" of the heterologous protein for assay simultaneously.

Presently preferred enhancing fusion partners comprise a detectable label: this permits one to verify that the fusion protein is actually expressed, to identify effectively transfected cells, and to sort enhanced cells apart from non-enhanced constructs. Other effective enhancing fusion partners can be identified through routine experimentation. For example, one can select a heterologous target protein that fails to produce a detectable (or convenient) phenotype in a selected host cell, but does produce a suitable phenotype when fused to GFP or lacZ (i.e., identifying a heterologous protein that is susceptible to enhancement). The target nucleic acid is then fused in frame to nucleic acids encoding candidate enhancing fusion partners, and the resulting fusions introduced into the host cells (or a panel of host cells). The resulting host cells are then assayed for a screenable phenotype, and successful enhancing fusion partners identified for later use. The fusion candidates can be drawn from any convenient source, including without limitation randomly-generated DNA, cDNA libraries, and randomly-sheared genomic DNA. However, it is presently preferred to select candidate fusion partners from the class of

polypeptides that includes useful proteinaceous labels and antigens. It is preferred to employ fusion partners that do not exhibit a phenotypic effect in the selected host cell when used alone, i.e., in the absence of a fusion partner. It is also presently preferred to select fusion partners whose folded structure is conformationally stable.

The phenotypic enhancement can be due to any of a variety of factors: (a) for molecules that require dimerization for activity, the fusion to an oligomeric fusion partner could stabilize the dimer; (b) the target protein may be stabilized with respect to degradation; (c) the fusion partner could help the protein fold more efficiently or stabilize its active structure; (d) the fusion partner may direct the fusion into a cellular compartment where the expressed protein creates the greatest degree of interference; or (e) it could facilitate binding of the protein to its substrate. Any or all of these factors can be present, depending on the target protein and the selected fusion partner.

One can verify that the phenotype is in fact due to an enhancement of the target protein activity by repeating the assay with a mutated target protein or fusion protein, wherein the active site of the target protein is disabled. If the active site(s) of the target protein is not already known, it can be determined using the techniques set forth in USSN 09/738,876. The loss of phenotype in the mutated fusion protein verifies that the activity is due in fact to the target protein. Alternatively, where there is a known inhibitor for the target protein activity, abrogation of the phenotype in the presence of an effective amount of the inhibitor confirms that the phenotype is due to the target protein activity.

In some instances, the fusion partner actually decreases the observed phenotype rather than increasing the phenotype. This can indicate that the target protein requires transport into certain subcellular compartments for activity, and that the transport is blocked by the presence of the fusion partner. Alternatively, a decrease in phenotype can indicate that the active site of the target protein is obscured by the fusion partner, that the fusion partner destabilizes the target protein structure or interferes with its folding or any oligomerization required for activity, or that the fusion protein is subject to accelerated degradation. If no difference in phenotype is detected, one can employ a different fusion partner.

Examples

The following examples are provided as a guide for the practitioner of ordinary skill in the art. Nothing in the examples is intended to limit the claimed invention. Unless otherwise specified, all reagents are used in accordance with the manufacturer's recommendations, and all reactions are performed at standard temperature and pressure.

Example 1

(Construction of an Enhanced Assay Cell)

(A) **PCR and Primers:**

This procedure is shown schematically in FIG. 1. The ORF was first cloned into a pARC expression vector under the control of the GAL1 promoter. This construct is typically sequenced and evaluated for growth. In order to add a C-terminal fusion to the ORF, we first polymerized both the ORF and the GAL1 promoter such that the ORF's stop codon was removed and an in-frame tail identical to the 5' end of the lacZ ORF (ATGACCATGATTACGGATTCACTG) was added. The ORF-specific 3' primer for this construct (which is the only new primer needed) reads as follows on the Watson strand: "~20ntORF(no stop)ATGACCATGATTACGGATTCACTG". Because this primer must correspond to the Crick strand we actually used its reverse-complement. The 5' PCR primer (which is a common primer) is also a composite of a 20nt substrate-specific section identical to the 5' end of the GAL1 promoter (used in our pARC vectors), GCATGCCGGTCACTAGAATG, and a 45nt tail that is identical to the new expression vector for the protein fusion pRS315lacZ or pRS425lacZ, CCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGC. The complete sequence of this common primer (JC573) is as follows: CCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGCATGCCGGTCACTAGAATG. One other common primer is for extending the 3' lacZ homology of the PCR product to 45nt. The sequence of this primer (AM685) is: ACGACGTTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTC.

Reactions were carried out in an MJ-research PTC-200 thermocyler, in 25 µl containing a diluted pARC expression construct, AM685 and JC573 primers (600 nM), ORF-specific primer (6 nM), 200 µM each dNTP and 0.1 units/µl Taq-plus precision polymerase (Stratagene) under the buffer conditions recommended by the supplier. Typical cycling conditions were as follows: (94°C/2min), 23×(94°C/30sec, 59°C/30sec, 72°C/1min), (72°C/10min), (4°C/"forever"). The

extension step at 72°C varied depending of the length of the template (usually we extended for 1 min per 1000 nt).

(B) **Expression Construct**:

Protein fusion expression vectors (FIG. 2) contain a short polylinker just before the lacZ ORF containing restriction sites for NheI, XhoI, PmeI and BamHI. The vector is gapped by restriction enzyme digestion at one or more of these sites.

PCR products are directly added to gapped expression vectors (pRS315lacZ or pRS425lacZ) for yeast transformation. Recombination into the vector is highly efficient and has been previously described (see, e.g., C.K. Raymond et al., Biotechniques (1999) 26(1):134-38, 140-41).

(C) **Analysis**:

A successful construct can be detected by monitoring GAL1-dependent lacZ expression. Colonies that express lacZ in 2% galactose but not in 2% glucose most likely have a plasmid that expresses a chimeric protein that starts with the heterologous ORF on the N-terminus and ends with the lacZ ORF on the C-terminus. Individual transformants and controls transformed with vector alone were inoculated into 200 µl of selective medium (SC-Leu, 2% glucose) in 96-well microtiter dishes and allowed to grow to saturation overnight at 30°C. The cultures were then diluted 1/400 in triplicate and into 100 µl of four SC-Leu media containing increasing amounts of galactose inducer (2% glucose, 2% raffinose + 0.02% galactose, and 2% raffinose + 0.2% galactose, 2% galactose) using a Beckman Multimeck 96 Automated 96-channel pipettor. Plates were incubated at 30°C wrapped in plastic to prevent edge effects from drying. Growth was monitored at approximately 24 h, 36 h and 48 h, though the ideal time depended on each particular strain and vector type. At each of these time points, plates were shaken vigorously to disperse the cells uniformly and immediately read in a Molecular Devices Spectra Max 340 plate reader. Results were shown as percent inhibition relative to the vector control, provided that the vector control itself grew to an OD_{600} of at least 0.1.

Percent inhibition = $100 \times (1 - (SiCu/CiSu))$,

where

Si - sample in induction medium (in 2% galactose)

Ci - control in induction medium

Su - sample in normal medium, "uninduced" (in 2% glucose)

Cu - control in normal medium

Example 2

(Phenotypic Enhancement)

The method described in Example 1 above was used to validate the integrity of the expressed heterologous ORFs. We noticed that the HIV protease-lacZ fusion constructs did not grow well in 2% galactose. This lead to the hypothesis that a protein fusion can sometimes exacerbate an ARC phenotype. We have measured the growth interference of most ARC constructs either as lacZ fusions or as native proteins and observed that the lacZ fusion could either enhance or decrease the interference phenotype (see FIG 3).

For HIV protease, the effect is seen both with a lacZ fusion and with a GFP fusion (see FIG. 4). Because GFP is a monomer the phenotypic enhancement for HIV protease is unlikely to be due to a stabilization of the protease dimer. Regardless, what is important is that the resultant phenotype is caused by the activity of the expressed heterologous protein. We have proven this point for HIV protease by testing both wild type HIV protease and an active site mutation D25A (See FIG. 5). Moreover, the growth interference phenotype can be reversed with a known inhibitor of HIV-protease (see FIG. 6).

CLAIMS

1.) A method for producing an assay cell having a screenable phenotype generated by a heterologous target gene in said assay cell, comprising:

a) introducing into a first host cell a first nucleic acid encoding a heterologous target protein operably connected to a promoter operable in said host cell;

b) obtaining expression of said heterologous target protein;

c) determining the degree to which said first host cell exhibits a screenable phenotype;

d) in the absence of a screenable phenotype, introducing into said host cell a second nucleic acid encoding a fusion protein comprising said heterologous target protein fused to an enhancing fusion partner, operably connected to a promoter operable in said host cell;

e) obtaining expression of said fusion protein;

f) determining the degree to which said host cell exhibits a screenable phenotype; and

g) selecting the host cell that exhibits a screenable phenotype to the greatest degree.

2. The method of claim 1, wherein said heterologous target protein comprises a plurality of heterologous target proteins.

3. The method of claim 2, wherein said plurality of heterologous target proteins comprises at least 10 different heterologous target proteins.

4. The method of claim 3, wherein said plurality of heterologous target proteins comprises at least 100 different heterologous target proteins.

5. The method of claim 4, wherein said plurality of heterologous target proteins comprises at least 1,000 different heterologous target proteins.

6. The method of claim 2, wherein each different heterologous target protein is introduced into a separate host cell.

7. The method of claim 1, wherein said enhancing fusion partner is selected from the group consisting of lacZ, GFP, and URA3.

8. The method of claim 1, wherein said first host cell comprises a plurality of different host cells.

9. A method for producing an assay cell having a screenable phenotypic response generated by a heterologous target gene in said assay cell, comprising:

a) introducing into a first host cell a first nucleic acid encoding a heterologous target protein operably connected to a promoter operable in said host cell, and introducing into a second host cell a second nucleic acid encoding a fusion protein comprising said heterologous target protein fused to an enhancing fusion partner, operably connected to a promoter operable in said host cell;

b) obtaining expression of said heterologous target protein and said fusion protein;

c) determining the degree to which said first host cell exhibits a screenable phenotype and the degree to which said second host cell exhibits a screenable phenotype; and

d) selecting the host cell exhibiting the greatest degree of screenable phenotype.

10. The method of claim 9, wherein said first host cell and said second host cell are substantially identical.

11. The method of claim 9, wherein said first host cell comprises a plurality of different host cells, and said second host cell comprises a plurality of different host cells substantially identical to said plurality of first host cells.

12. The method of claim 9, wherein said first nucleic acid comprises a plurality of different nucleic acids, each encoding a different heterologous target protein.

13. The method of claim 12, wherein said plurality of different nucleic acids comprises at least 10 different nucleic acids.

14. The method of claim 13, wherein said plurality of different nucleic acids comprises at least 100 different nucleic acids.

15. The method of claim 9, wherein said enhancing fusion partner is selected from the group consisting of lacZ, GFP, and URA3.

16. A process for identifying compounds capable of inhibiting the biological activity of a target protein, said process comprising:

a) providing a first host cell comprising a first nucleic acid encoding a heterologous target protein operably connected to a promoter operable in said host cell, and providing a second host cell comprising a second nucleic acid encoding a fusion protein comprising said heterologous target protein fused to an enhancing fusion partner, operably connected to a promoter operable in said host cell;

b) obtaining expression of said target protein and said fusion protein;

c) determining the degree to which said first host cell exhibits a screenable phenotype and the degree to which said second host cell exhibits a screenable phenotype;

d) selecting the host cell exhibiting the greatest degree of screenable phenotype;

e) contacting the selected host cell with a candidate compound;

f) determining the degree to which the screenable phenotype of said selected host cell is modulated by the presence of said candidate compound; and

g) selecting a candidate compound that reverses said screenable phenotype to a measurable degree.

17. The process of claim 16, wherein said first host cell and said second host cell each comprise a plurality of different host cells strains.

18. The process of claim 17, wherein the host cell exhibiting the greatest degree of screenable phenotype is selected for each host cell strain, to form a panel of selected host cells.

19. The process of claim 16, wherein said target protein comprises a plurality of target proteins.

20. The process of claim 19, wherein a host cell exhibiting the greatest degree of screenable phenotype is selected for each different target protein, to form a panel of target protein assay cells.

21. The process of claim 20, wherein said first host cell and said second host cell each comprise a plurality of different host cells strains.

22. The process of claim 21, wherein the host cell exhibiting the greatest degree of screenable phenotype is selected for each host cell strain, to form a panel of selected host cells.

23. The process of claim 16, wherein said enhancing fusion partner is selected from the group consisting of lacZ, GFP, and URA3.

24. The process of claim 16, wherein said enhancing fusion partner comprises a plurality of different enhancing fusion partners.

25. The process of claim 16, wherein said candidate compound comprises a plurality of candidate compounds.

26. An assay panel, comprising:

a plurality of different first host cells, each first host cell comprising a first nucleic acid encoding a heterologous target protein operably connected to a promoter operable in said host cell, and a plurality of second host cells, each second host cell corresponding to a first host cell and comprising a second nucleic acid encoding a fusion protein comprising said heterologous target protein fused to an enhancing fusion partner, operably connected to a promoter operable in said host cell, wherein for each target protein at least one of said first host cell and said second host cell which expresses said target protein exhibits a screenable phenotype.

27. The assay panel of claim 26, wherein said heterologous target protein comprises a plurality of heterologous target proteins.

28. The assay panel of claim 26, wherein said enhancing fusion partner is selected from the group consisting of lacZ, GFP, and URA3.

Fig1. Construction on LacZ C-terminal protein fusions

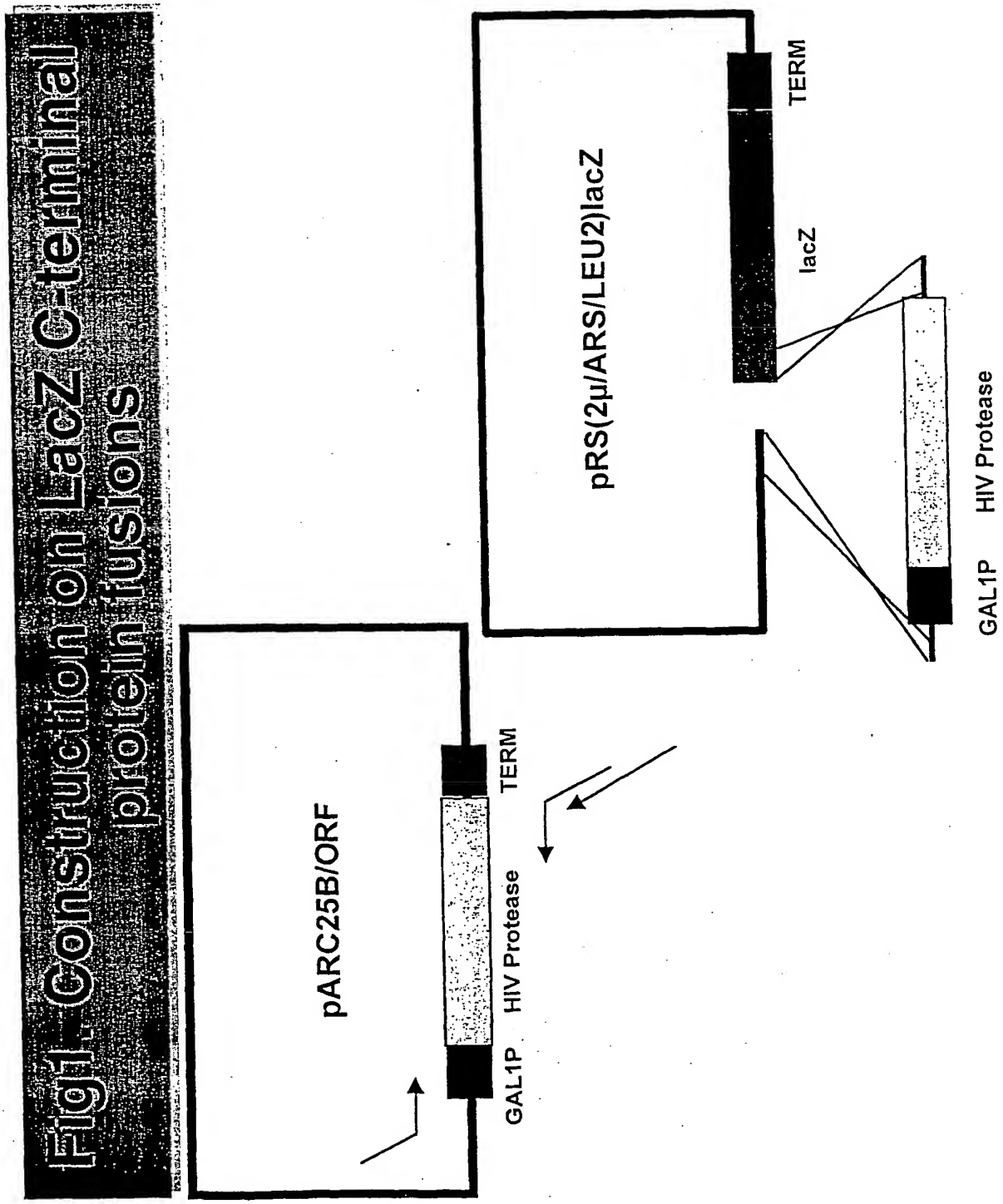


Fig2: pRS315_lacZ

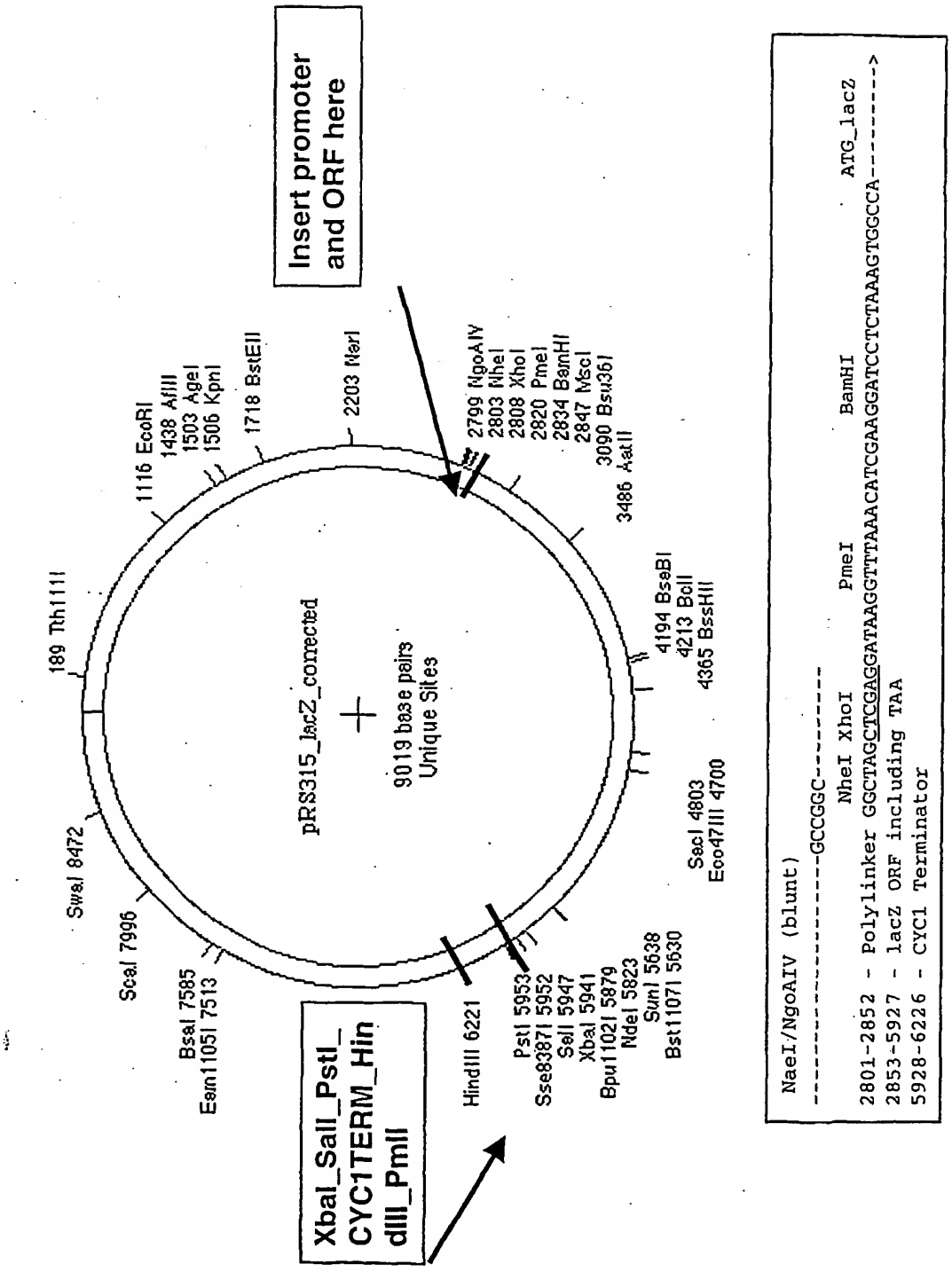


Fig 3. Influence C-terminal lacZ fusion tag on interference phenotypes

ORF	Native	Fusion
RHINO_2A	2micron 94	2micron 80 CEN
HPV_E1	2micron 92	2micron pRS315
INFL_M2	2micron 91	2micron 69 2micron
HBV_ROL	2micron 89	2micron 92 CEN
HIV_VPR	2micron 84	2micron 69 CEN
HPV_E2	2micron 83	2micron 55 pRS315
INFL_NS1	2micron 80	2micron 2micron
VZV_UL33	2micron 80	2micron 2micron
INFL_PB2	2micron 70	2micron 89 2micron
RHINO_3ABC	2micron 70	2micron 2micron
CMV_E2	2micron 67	2micron 2micron
INFL_NS2	2micron 65	2micron 87 CEN
INFL_PB1	2micron 57	2micron 53 2micron
INFL_NP	2micron 54	2micron 94 2micron
HCV_CORE	2micron 53	2micron 2micron
INFL_PA	2micron 53	2micron 2micron
HIV_REV	2micron 48	2micron 2micron
RHINO_2BC	2micron 46	2micron 2micron

ORF	Native	Fusion
HBV_X	2micron 74	2micron 50 2micron
HBV_TP	2micron 70	2micron 93 CEN
HIV_VPU	2micron 72	2micron 64 2micron
HSV_UL26	2micron 72	2micron 72 2micron
HIV_VIF	2micron 58	2micron 55 2micron
HIV_NEF	2micron 56	2micron 60 2micron
HPV_E6	2micron 56	2micron 100 2micron
HIV_PRO	2micron 52	2micron 97 INT
CMV_UL80	2micron 38	2micron 21 2micron
HPV_E4	2micron 30	2micron 39 CEN
RSV_N	2micron 30	2micron 30 2micron
RSV_NS1	2micron 29	2micron 39 2micron
RSV_P	2micron 25	2micron 50 2micron
CMV_UL97	2micron 15	2micron 15 2micron
RSV_NS2	2micron 10	2micron 20 2micron
RSV_MATRIX	2micron 10	2micron 20 2micron
RSV_F_ORF2	2micron 5	2micron 50 2micron
RSV_M_ORF2	2micron 3	2micron 50 2micron

Fig.4 HIV_PR-GFP fusions expressed in pARC25B in EIS20-2B Percent inhibition at three time points

	24h	28h	32h
HIV_PR WT (avrg)	87	92	93
stdev	0.25	0.87	0.87
HIV_PR D25A (avrg)	31	26	15
stdev	4.73	4.45	5.34

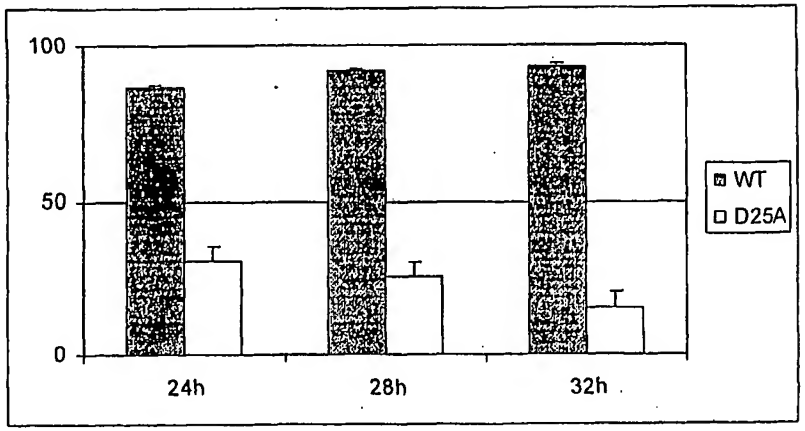


Fig 5. ABC Phenotype Enhancement

Constructs	2μ	CEN
Native HIV Protease	44%	0%
Active Mutant HIV Protease (D25A)	34%	NA
HIV Protease-LacZ fusion	84%	84%
Mutant HIV Protease (D25A)-LacZ fusion	38%	0%

Fig.6A HIV Protease/LacZ fusion Titration with Known Inhibitor

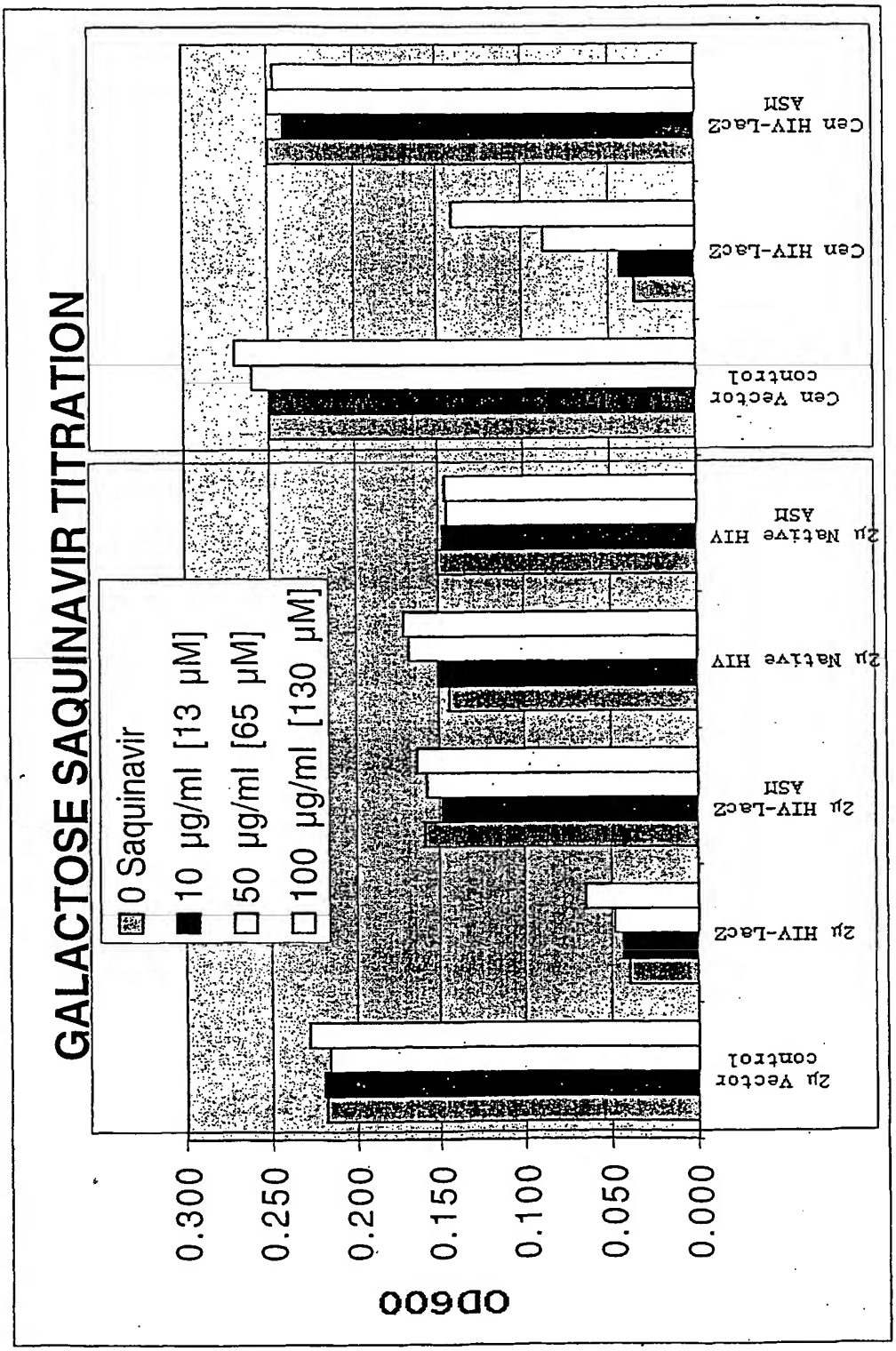
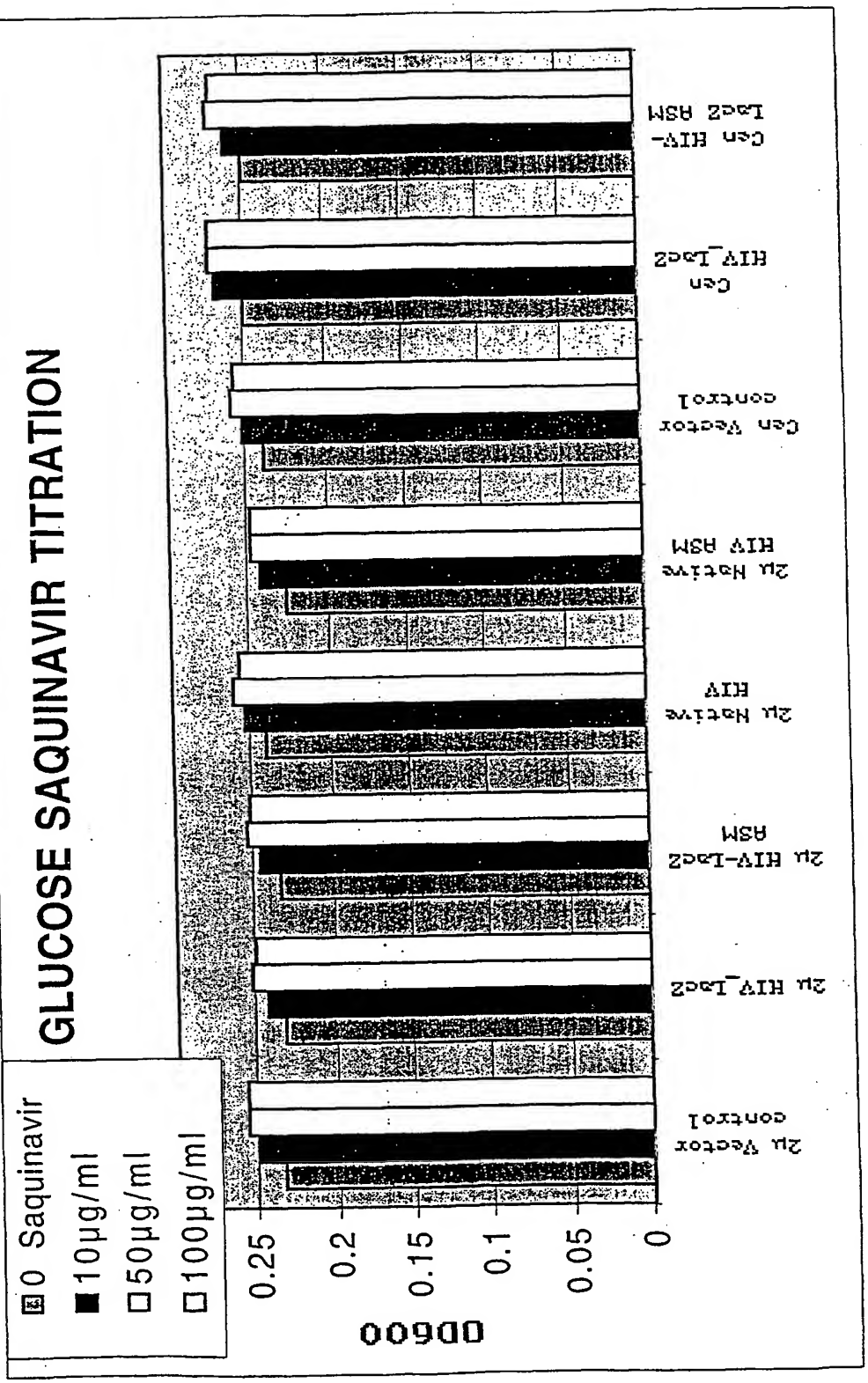


Fig 6B HIV Protease/LacZ Fusion SaquinaVir Titration



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/02857

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12P 21/00, C12N 5/02
US CL : 435/69.7, 70.1, 325
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/69.7, 70.1, 325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99/40227 A1 (THE UAB RESEARCH FOUNDATION) 12 August 1999 (12.08.1999), see entire document.	1-28
Y	US 5.871,934 A (LEE et al.) 16 February 1999 (16.02.1999), see entire document.	1-28
Y	WO 01/04331 A2 (CALGENE LLC) 18 January 2001 (18.01.2001), see entire document.	1-28
Y	WO 99/27365 A1 (TULARIK INC..) 03 June 1999 (03.06.1999), see entire document.	1-28

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Date of the actual completion of the international search
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/02857

Continuation of B. FIELDS SEARCHED Item 3:
APS STN MEDLINE CAPLUS BIOSIS
Search terms: pharmaceutical screen, drug screen, fusion protein, green fluorescent protein, lacZ, galactosidase, ura3, stabiliz?

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